Title	Aromatic prenylation in the biosynthesis of bitter acids of hop (Humulus lupulus L.).
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Citation	Sustainable humanosphere : bulletin of Research Institute for Sustainable Humanosphere Kyoto University (2013), 8: 19-20
Issue Date	2013-02-01
URL	http://hdl.handle.net/2433/182601
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

ABSTRACTS (PH D THESIS)

Aromatic prenylation in the biosynthesis of bitter acids of hop (*Humulus lupulus* L.).

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Hop (*Humulus lupulus* L., Cannabinaceae) is a perennial and dioecious climbing plant and female plants of the species are cultivated world-wide for use as an essential ingredient of beer. Female flowers, also called hop cones, give the characteristic flavor and bitter taste to beer due to a variety of essential oils and aromatic compounds, which are biosynthesized and accumulated exclusively in yellow glandular trichomes, also designated lupulins, which develop at the basal part of hop cone bracts. Among the secondary metabolites produced by the hop plant, prenylated acylphloroglucinols, conventionally called 'bitter acids', have received a large amount of attention because their characteristic bitter property is important for beer taste; moreover, their divergent biological activities, including radical scavenging activity, angiogenesis inhibition, and inducing effect for P450 enzyme, are beneficial for human health. Hop cones also contain prenylated flavonoids, among which the major one is xanthohumol, a prenylated chalcone derivative, which has potential applications as a cancer chemopreventive agent. The proposed biosynthetic pathway of bitter acids in hops, also called α- and β-acid (humulone and lupulone, respectively), are shown in Fig. 1, with the biosynthesis of xanthohumol illustrated in parallel. In the biosynthesis of these secondary metabolites in hops, aromatic prenyltransferases play a crucial role for both phloroglucinol and flavonoid derivatives. The plant prenyltransferases that recognize aromatic secondary metabolites are a new topic of research in plant molecular biology; the first flavonoid-specific prenyltransferase, naringenin 8-dimethyallyltransferase (SfN8DT), was identified in 2008, and thereafter a pterocarpan and isoflavonone-specific prenyltransferases, glycinol 4-dimethylallyltransferase (G4DT) and genistein 6-dimethylallyltransferase (SfG6DT), respectively, have been reported. These enzymes are all divalent cation-requiring membrane-bound proteins, and those characterized to date have been localized in plastids.

In this study, we constructed a cDNA library from the lupulin gland-rich portion of female flower bracts, and randomly sequenced 11,233 EST clones, obtaining sequence information for 6613 non-redundant ESTs. Among them, a cDNA designated Humulus lupulus prenyltransferase-1 (HlPT-1) was a candidate for the gene coding for prenylation enzyme as it possessed three features of the plant aromatic prenyltransferase family, namely, a D-rich motif, multiple membrane-spanning domains, and a putative transit peptide sequence at the N-terminus. Indeed, HlPT-1 was highly expressed in hop cones, especially in the lupulin glands. Moreover, a GFP fusion experiment showed that the transit peptide of HlPT-1 actually localized the GFP fusion protein to plastids in a manner similar to other flavonoid prenyltransferases in the legume plants. Subsequently, we heterologously expressed HlPT-1 protein in insect cells and demonstrated its enzymatic function in vitro assays using phloroglucinol derivatives and various flavonoids as prenyl acceptor substrates in the presence of dimethylallyl diphosphate as a prenyl donor.

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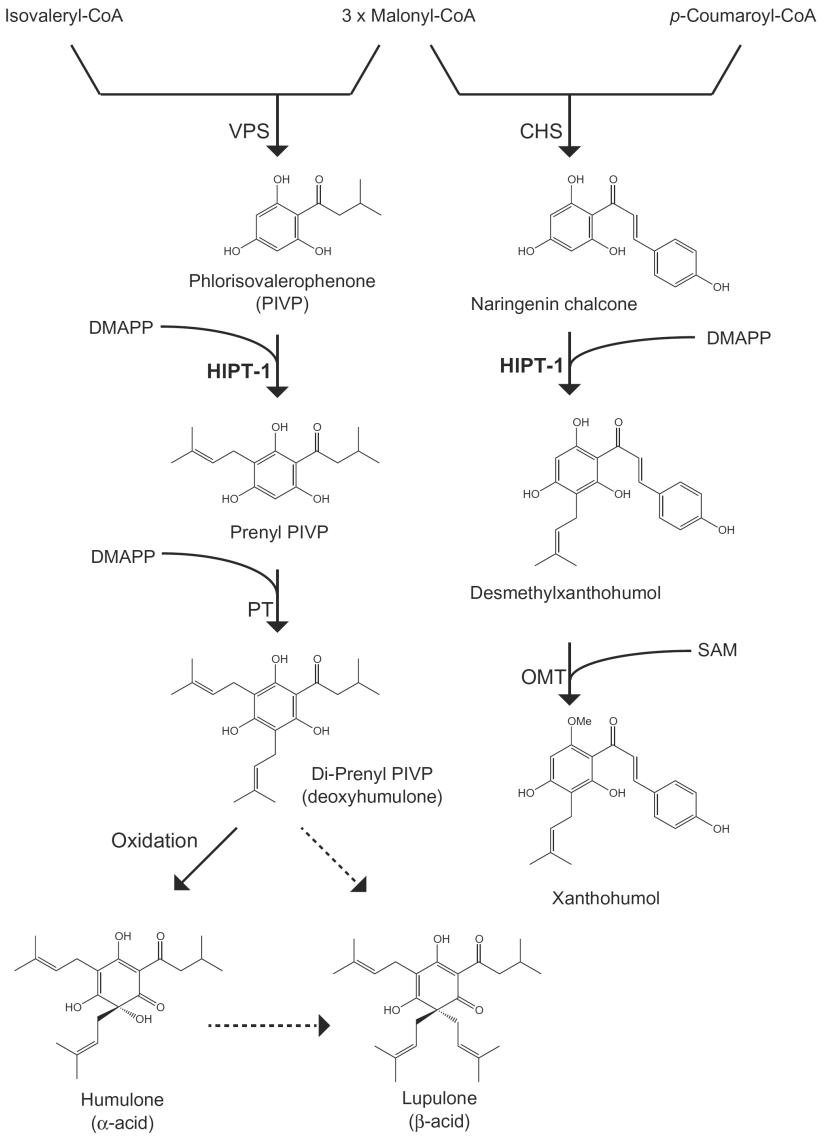

Fig. 1. Biosynthesis of bitter acids (humulone and lupulone) and xanthohumol in lupulin glands of hop.
DMAPP, dimethylallyl diphosphate; VPS, valerophenone synthase; PT, prenyltransferase; CHS, chalcone synthase; OMT, *O*-methyltransferase; SAM, *S*-adenosylmethionine. (modified from Tsurumaru, Y., et al. BBRC, 417, 393-398, 2012)

The HlPT-1 identified in hops catalyzes the first step in prenylation of aromatic substances, adding dimethylallyl moiety to phloroglucinol derivatives and leading to the formation of humulone and lupulone derivatives. HlPT-1 is also responsible for the formation of xanthohumol by transferring prenyl residue to chalcone. Here, we found that HlPT-1 has many characteristic features as an aromatic prenyltransferase that set it apart from other reported members; namely, narrow optimum pH at around neutral pH, sharp preference for Mg^{2+} as a divalent cation, and broad substrate specificity. It is likely that, HlPT-1 recognizes a phloroglucinol portion as the prenyl acceptor, which is a common structure for the A-ring of naringenin chalcone.